

RESEARCH PAPER

Comparative effect of partial root-zone drying and deficit irrigation on incidence of blossom-end rot in tomato under varied calcium rates

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Abstract

This study investigated the comparative effects of reduced irrigation regimes—partial root-zone drying (PRD) and conventional deficit irrigation (DI)—on the incidence of blossom-end rot (BER) in tomato (*Solanum lycopersicum* L.) under three Ca-fertilization rates: 0, 100, and 200 mg Ca kg⁻¹ soil (denoted Ca0, Ca1, and Ca2, respectively). The plants were grown in split-root pots in a climate-controlled glasshouse and treated with PRD and DI during early flowering to the fruit maturity stage. The results showed that, in comparison with DI treatment, PRD significantly reduced BER incidence. A greater xylem sap abscisic acid concentration, lower stomatal conductance, and higher plant water status in the PRD in relation to the DI plants might have contributed to the increased fruit Ca uptake, and could have reduced BER development in tomato fruits. Therefore, under conditions with limited freshwater resources, application of PRD irrigation could be a promising approach for saving water and for preventing BER development in tomatoes.

Key words: ABA, BER, calcium, plant water potential, stomatal conductance, xylem sap.

Introduction

Blossom-end rot (BER) is a physiological disorder in tomato (*Solanum lycopersicum* L.) fruit that may reduce the marketable yield by up to 50% (Taylor *et al.*, 2004). Although a high rate of BER occurrence has often been associated with low calcium (Ca) content in the fruit tissue (Ho and White, 2005), total tissue Ca content is not a precise predictor of BER development, and the Ca content for fruit with BER symptoms may be equal to or higher than that for healthy fruit (Nonami *et al.*, 1995; Saure, 2001). None the less, increasing fruit Ca uptake via direct Ca sprays has been shown to effectively reduce the incidence of BER in tomatoes (Wada *et al.*, 1996; Ho, 1999; Schmitz-Eiberger *et al.*, 2002); likewise, manipulation of growth conditions such as increasing air humidity that decrease leaf transpiration rate may promote

fruit Ca uptake and reduce the risk of BER development (Li *et al.*, 2001). Additionally, as the rate of fruit Ca uptake is almost exclusively dependent on the abundance of functional xylem vessels connecting to the fruits, tomato genotypes possessing a stronger xylem network have been found to be less susceptible to BER (Ho *et al.*, 1993). Most recently, Tonetto de Freitas *et al.* (2011) reported that exogenous abscisic acid (ABA) application could reduce leaf transpiration rate and enhance the xylem connection to the fruits, thus increasing fruit Ca uptake and preventing BER development in tomatoes. Accordingly, these authors suggested that exogenous application of ABA could be used as a potential tool for commercial prevention of BER in greenhouse tomato production (Tonetto de Freitas *et al.*, 2011). However, as the

Abbreviations: Ψ_l , Midday leaf water potential; Ψ_r , root water potential; ABA, abscisic acid; [ABA]_{xylem}, ABA concentration in the xylem sap; ANOVA, analysis of variance; BER, blossom-end rot; Ca, calcium; DI, deficit irrigation; g_s , stomatal conductance; PRD, partial root-zone drying; SE, standard error of the mean.
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concentration of the ABA used in the study was extremely high and economically infeasible for current commercial practice, exploitation of the plant potential to enhance the endogenous ABA level could be a more effective approach to prevent BER development in tomatoes.

Apart from genetic modification for enhancing plant ABA production (Thompson *et al.*, 2007; Tung *et al.*, 2008), field management practices such as partial root-zone drying (PRD) and deficit irrigation (DI) strategies, which expose the plant to moderate drought stress, could also increase ABA levels in the plants. Numerous studies have demonstrated that a greater concentration of ABA in the xylem sap of plants under PRD and DI treatments induces partial stomatal closure and decreases leaf expansion growth, thereby curtailing plant water use and improving water use efficiency (Dodd, 2009; Liu *et al.*, 2009). In addition, accumulated evidence has indicated that, given the same irrigation volume, PRD frequently results in significantly higher ABA concentration in the xylem than DI, which has been considered to contribute to better stomatal control over plant water use (Dodd *et al.*, 2008; Wang *et al.*, 2010). Although there is a general consensus that tomato fruits are hydraulically isolated from the xylem stream and therefore may be inaccessible for the root-sourced ABA signalling, particularly at later stages of fruit development (Davies *et al.*, 2000), studies have demonstrated that, during the early stage of fruit growth, soil water deficit treatment or exogenous ABA application could stimulate xylem development in the fruits (Davies *et al.*, 2000; Tonetto de Freitas *et al.*, 2011). Despite the possible effect of ABA in reducing BER incidence, previous studies have indicated that low plant water status is associated with a low number of functional xylem vessels in tomato fruits (Belda and Ho, 1993), and reduced irrigation increases BER occurrence in tomatoes (Adams and Ho, 1993; Taylor *et al.*, 2004). Consistent with this, Zegbe *et al.* (2007) found that, compared with the full irrigation control, both PRD and DI increased BER incidence in drip-irrigated tomatoes. None the less, several studies have noted that, compared with DI, PRD plants often possess lower stomatal conductance and higher leaf water status (Xu *et al.*, 2009; Wang *et al.*, 2010). Moreover, our recent work has indicated that PRD increases Ca concentration in the xylem of tomatoes at the early fruiting stage (Wang *et al.*, 2012), indicating that the root Ca uptake is enhanced in PRD treatment. However, whether these effects of PRD on plant water status and root Ca uptake could bring about a reduced BER incidence compared with DI treatment in tomato fruits remains unknown. As freshwater resources become scarcer worldwide and in many regions full irrigation practice that compensates 100% of crop evapotranspiration becomes inapplicable, comparing the effect of different reduced irrigation regimes such as PRD and DI on crop performance will be of strategic significance.

In this study, tomato plants were grown under three Ca-fertilization rates and exposed to PRD and DI treatments from flowering to fruit maturity stages. The effects of the reduced irrigation regimes in combination with the Ca-fertilization rates on leaf and root water status,

stomatal conductance, xylem sap ABA and ionic concentrations, K, Ca, and Mg contents in the fruits, leaves, stems, and roots, and their partitioning among these plant organs were investigated. The objective was to examine whether any of the effects induced by the PRD treatment could reduce the incidence of BER in tomato fruits in comparison with the DI treatment. It is anticipated that the results obtained here will provide deeper insights into the mechanisms controlling BER development, and will be helpful in formulating management practices preventing this physiological disorder, thereby improving fruit yield and quality in tomatoes.

Materials and methods

Experimental setup

The experiment was conducted from January to April 2012 in a climate-controlled glasshouse at the experimental farm of the Faculty of Science, University of Copenhagen, Taastrup, Denmark. At the fifth-leaf stage, tomato (*Solanum lycopersicum* L. var. Cedrico) seedlings were transplanted into 10 l pots. The pots were divided vertically into two equal-sized compartments with plastic sheets such that water movement between the two compartments was prevented. The pots were filled with 14.0 kg of naturally dried soil with a bulk density of 1.36 g cm⁻³. The soil was taken from a nutrient-depletion plot of a long-term soil fertilization experiment located near the experimental farm. The texture of the soil was sandy loam with a pH of 7.4, total C 12.7 g kg⁻¹, total N 1.4 g kg⁻¹, total P 22 mg kg⁻¹, exchangeable Ca 3.0 mmol kg⁻¹, and exchangeable K, Mg, and Na <1.0 mmol kg⁻¹. The soil was sieved by passing through a 5 mm mesh before filling the pots. The soil had a volumetric soil water content (vol. %) of 30.0 and 5.0% at water-holding capacity and permanent wilting point, respectively. The climatic conditions in the glasshouse were set at: 20/17±2 °C day/night air temperature, 16 h photoperiod and >500 μmol m⁻² s⁻¹ photosynthetic active radiation supplied by sunlight plus metal-halide lamps.

Ca-fertilization and irrigation treatments

Three Ca-fertilization rates, namely 0, 100, and 200 mg Ca kg⁻¹ soil (denoted Ca0, Ca1, and Ca2, respectively), were used in the experiment. The Ca fertilizer supplied as Ca(NO₃)₂·4H₂O was mixed thoroughly with the soil before filling the pots. For each pot, 3.0 g N, 0.87 g P, 4.0 g K, and 1.68 g Mg were also applied as Mg(NO₃)₂·6H₂O, KH₂PO₄, KNO₃ and MgSO₄·7H₂O into the soil to meet the nutrient requirements for plant growth. The tomato plants were well watered in the first 3 weeks after transplanting. Thereafter, the plants were subjected to two reduced irrigation regimes: (i) PRD irrigation, in which half of the root system was watered daily at 16:00 h to a water content of 28%, whilst the other half was allowed to dry until the water content decreased to 10%, and then the irrigation was switched between the two soil compartments; and (ii) DI, in which the same amount of water used in PRD was irrigated evenly in the two soil compartments. The water used for manual irrigation was tap water with negligible concentrations of nutrients (except for Ca). The experiment was a complete factorial design comprising six treatments and each treatment had four replicates (one plant per replication). The reduced irrigation regimes lasted for 8 weeks during which each compartment of the PRD pots experienced four drying and rewetting cycles. The average soil water content in the pot was monitored by a time domain reflectometer (TDR, TRASE, Soil Moisture Equipment, CA, USA) with probes (35 cm in length) installed in the middle of each soil compartment. The changes in

soil water content in the pots during the treatment period will be presented elsewhere (Sun *et al.*, unpublished).

Measurements

Stomatal conductance (g_s) of the upper-canopy fully expanded leaves was measured using a leaf porometer (Decagon Devices, Pullman, WA, USA) five times during the experimental period (i.e. at 0, 2, 4, 6, and 8 weeks after onset of treatments). The measurements were conducted from 10:00 to 12:00 am, and at each measurement, g_s of both abaxial and adaxial leaf surfaces of three leaves per plant was determined. The total g_s of the leaf was computed as the sum of g_s values on the abaxial and adaxial leaf surfaces. After determination of g_s , midday leaf water potential (Ψ_l) was determined using a pressure chamber (Soil Moisture Equipment). In order to examine the overall effects of the irrigation and Ca-fertilization treatments on g_s and Ψ_l , here only the mean values of the five measurements obtained at different dates are presented.

By the end of the treatment period (i.e. 56 d after onset of the irrigation treatments), all plant materials were harvested. Xylem sap was collected by pressurizing the roots of the potted plants in a Scholander-type pressure chamber. The entire pot was sealed into the chamber and the shoot was detopped at 5–10 cm from the stem base. With the stem stump protruding outside the chamber, pressure was applied slowly until the root water potential (Ψ_r) was equalized. The cut surface was cleaned with pure water and dried with blotting paper. The pressure was increased gradually until it equalled leaf water potential of the plants in order to obtain a sap flow rate similar to the whole plant transpiration rate to avoid dilution effects (Dodd *et al.*, 2008). However, as the hydraulic resistance of the shoot was removed, the flow rate could be higher than the actual transpiration rate, and thus the ABA concentration in the sap ($[ABA]_{xylem}$) might have been underestimated. Xylem sap (1.5 ml) was collected using a pipette from the cutting surface into an Eppendorf vial wrapped with aluminium foil. All sap samples were frozen immediately in liquid nitrogen after sampling and stored at –80 °C until analysis. $[ABA]_{xylem}$ was determined by ELISA using the protocol of Asch (2000).

K^+ , Ca^{2+} , and Mg^{2+} concentrations in the xylem sap were analysed by ion chromatography (Metrohm AG, Herisau, Switzerland) using on a Metrosep C4-100 analytical column (4 × 125 mm, 1.7 mM nitric acid/0.7 mM dipicolinic acid eluent) (Wang *et al.*, 2012).

At harvesting, plant samples were divided into leaves, stem, and fruits. The entire root system of the plant was collected after washing. The dry biomass of plant samples was determined after oven drying at 70 °C until constant weight. The dry samples were thoroughly grounded to a fine powder. Approximately 1 g of dry plant material was weighed out and added to a crucible. The samples were ashed at 500 °C in an electric muffle furnace (Controller B180, Nabertherm GmbH, Lilienthal/Bremen, Germany) for 8 h. After cooling to room temperature, 5 ml of 3 M HCl was added to each crucible and the crucible was then placed on a sand bath at 120 °C and evaporated for 2–3 h to dryness. The crucible was removed from the sand bath and cooled to room temperature. To each crucible, 10 ml of 1 M HNO₃ was added to dissolve the ash. After complete stirring, the solution was transferred to a 50 ml volumetric flask. Thereafter, the crucible was washed several times with double-deionized water, all of which was also transferred into the 50 ml volumetric flask. The samples were then filtered into 50 ml tubes and kept at 4 °C until analysis. The contents of minerals including K, Ca, and Mg of the plant samples were determined by an Atomic Absorption Spectrometer (Perkin-Elmer 3300, Norwalk, CT USA).

Statistical analysis

The data were analysed by two-way analysis of variance (ANOVA) using SAS 8.2 (SAS Institute). Duncan's multiple range test was used to assess the differences between treatments at a significant level of 5%. Regression analyses were used to determine the relationships among the measured variables.

Results

Effects of reduced irrigation regimes and Ca-fertilization rates on Ψ_l , Ψ_r , g_s , and $[ABA]_{xylem}$ in tomatoes

Results of a two-way ANOVA indicated that Ca-fertilization rates had not significantly affected Ψ_l , Ψ_r , g_s , and $[ABA]_{xylem}$, whereas the reduced irrigation regimes had significant effects on Ψ_r , g_s , and $[ABA]_{xylem}$, and with a clear tendency to influence Ψ_l in tomato plants (Fig. 1). Across the three Ca-fertilization rates, PRD plants had significantly higher Ψ_r

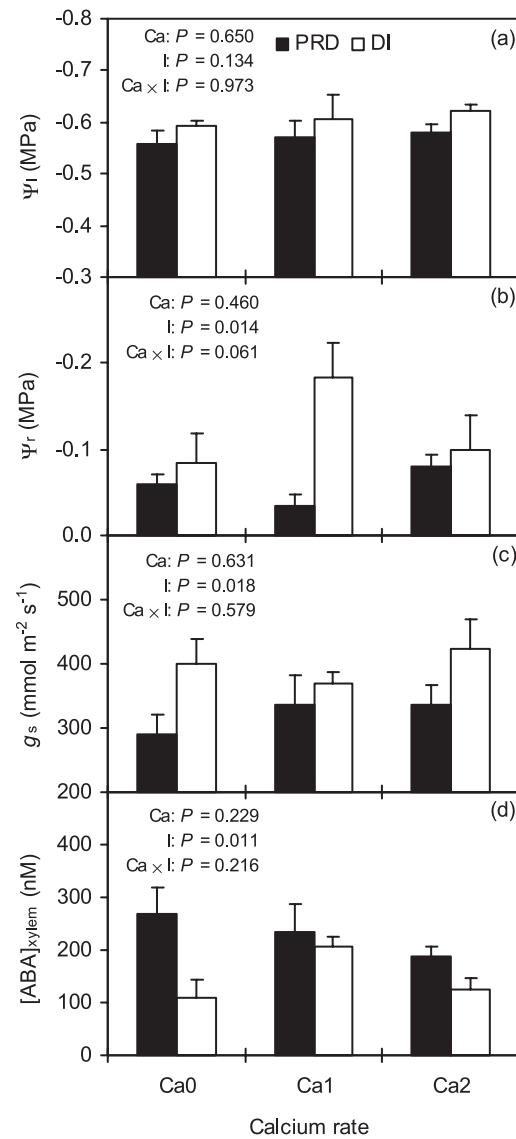


Fig. 1. Midday leaf water potential (Ψ_l) (a), root water potential (Ψ_r) (b), stomatal conductance (g_s) (c), and xylem sap ABA concentration ($[ABA]_{xylem}$) (d) of tomato plants as influenced by reduced irrigation regimes (PRD and DI) and Ca-fertilization rates (Ca0, Ca1, and Ca2). Statistical comparisons (two-way ANOVA) between the irrigation and Ca-fertilization treatments as well as their interactions (Ca-fertilization, Ca; irrigation, I; and the interaction, CaxI) are presented for each variable. Error bars indicate standard error of the mean (SE) ($n=4$).

and $[ABA]_{xylem}$ and slightly higher Ψ_1 but significantly lower g_s than DI plants (Fig. 1). A significant negative correlation between g_s and $[ABA]_{xylem}$ was observed across all treatments (Fig. 2).

Effects of reduced irrigation regimes and Ca-fertilization rates on K^+ , Ca^{2+} , and Mg^{2+} concentrations in the xylem sap of tomatoes

The concentrations of K^+ , Ca^{2+} , and Mg^{2+} in the xylem sap were not affected by the reduced irrigation regimes but were significantly influenced by the Ca-fertilization rates. Across the two reduced irrigation regimes, Ca1 plants had the highest concentrations of the three cations, followed by Ca0, with the lowest for Ca2. There was no significant effect of the interactions between the reduced irrigation regimes and Ca-fertilization rates on the concentration of the three cations in the xylem sap (Table 1).

BER incidence and BER fruit yield percentage as affected by reduced irrigation regimes and Ca-fertilization rates

Fig. 3 shows the effects of PRD and DI in combination with three Ca-fertilization rates on BER incidence and BER fruit yield percentage of tomato plants. It was found that Ca-fertilization rate did not significantly influence the number and yield of BER and non-BER fruits as well as the BER incidence and BER fruit yield percentage, whereas the reduced irrigation regimes significantly affected BER fruit yield and BER fruit yield percentage, as they were significantly lower in PRD than in DI plants (Fig. 3d, f). In addition, there was a clear indication that PRD led to lower BER fruit number and

BER incidence but higher non-BER fruit number compared with the DI treatment (Fig. 3a–c).

Influences of reduced irrigation regimes and Ca-fertilization rates on K, Ca, and Mg content in BER and non-BER fruits

The output of the two-way ANOVA indicated that neither irrigation regimes nor the Ca-fertilization rates significantly affected K, Ca, and Mg content in BER and non-BER fruits (Fig. 4 and Table 2). However, when comparing the mineral contents between the BER and non-BER fruits, it was found that BER fruits had significantly lower Ca and Mg content in relation to non-BER fruits, whilst K content was identical for the two kinds of fruit (Fig. 4). The results also showed that, irrespective of the reduced irrigation regime and Ca-fertilization rate, fruit K content ranged from 27 to 32 mg g⁻¹, which was significantly greater than the fruit Ca and Mg content (~0.8–1.5 and 1.2–1.5 mg g⁻¹ for Ca and Mg, respectively).

K, Ca, and Mg content in tomato leaves, stem, and roots as affected by reduced irrigation regimes and Ca-fertilization rates

K content varied significantly in the plant vegetative organs and was highest in the leaves (25–30 mg g⁻¹), intermediate in the stem (15–20 mg g⁻¹), and lowest in the roots (~5 mg g⁻¹) (Fig. 5a). Unlike K content, Ca and Mg content was significantly higher in the leaves and roots compared with that in the stem (Fig. 5b, c). The reduced irrigation regimes significantly affected root K and Ca content, which were significantly higher for DI than for PRD (Fig. 5a, b and Table 2). In addition, DI plants had a significantly greater leaf Mg content than the PRD plants (Fig. 5c and Table 2). No significant effects of Ca-fertilization rate or interactions between irrigation regimes and Ca-fertilization rates on any of the above variables were observed (Table 2).

Partitioning of K, Ca, and Mg into the fruits, leaves, stem, and roots as affected by reduced irrigation regimes and Ca-fertilization rates

The partitioning of K, Ca, and Mg among plant organs in tomato as affected by PRD and DI in combination with the three Ca-fertilization rates is presented in Fig. 6. It was apparent that the partitioning of the three minerals varied dramatically among the plant organs and the patterns of partition differed among the minerals. For instance, more than 50% of K was partitioned in fruits, whilst that for Mg was 10–15% and for Ca was only 2% (Fig. 6). Only the reduced irrigation regimes significantly affected mineral elements partitioning among the plant organs (Table 3). Compared with the DI plants, PRD plants allocated significantly greater amounts of K, Ca, and Mg into the stem, and significantly higher levels of Ca and Mg into the fruits but less Ca into the roots (Fig. 6 and Table 3). Ca-fertilization rates and the interactions between the irrigation regimes and Ca-fertilization rates had no effect on any of the above variables (Table 3).

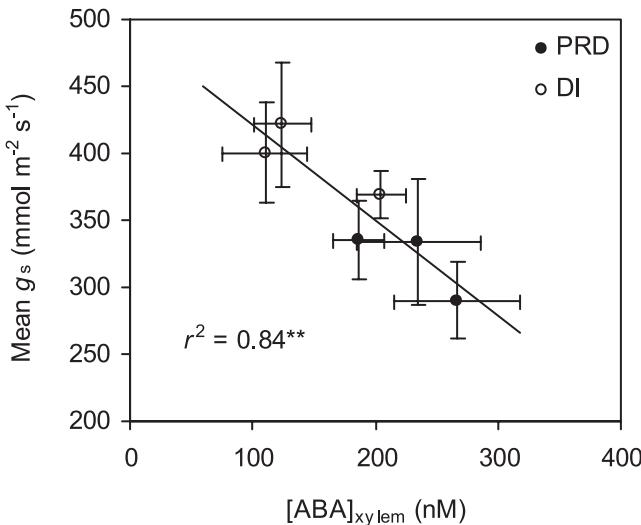


Fig. 2. Relationship between stomatal conductance (g_s) and xylem sap ABA concentration ($[ABA]_{xylem}$) of tomato plants under different irrigation regimes (PRD and DI) and Ca-fertilization rates (Ca0, Ca1, and Ca2). ** indicates the significance of the regression line at $P < 0.01$. Error bars indicate SE ($n=4$).

Table 1. Output of the two-way ANOVA for K^+ , Ca^{2+} , and Mg^{2+} concentration ($mol\ m^{-3}$) in the xylem sap of tomato plants as affected by the irrigation regimes (PRD and DI) and Ca-fertilization rates (Ca0, Ca1, and Ca2).

Mineral	Irrigation treatment			Ca-fertilization rate				P
	PRD	DI	P	Ca0	Ca1	Ca2	P	
K^+	17.9	19.8	NS	20.1a	21.9a	14.5b	*	NS
Ca^{2+}	7.7	8.5	NS	8.2ab	9.9a	6.4b	*	NS
Mg^{2+}	4.1	4.5	NS	4.2ab	5.4a	3.3b	*	NS

*Significant at $P < 0.05$, indicated by different letters; NS, not significant.

Relationships between BER incidence and fruit Ca content, fruit Mg:Ca content ratio, and fruit K:Ca content ratio

Regression analyses indicated that there was a close negative linear relationship between BER incidence and the weighted average Ca content in the fruits (Fig. 7a), whilst positive linear relationships between BER incidence and fruit Mg:Ca ratio and

fruit K:Ca ratio were found in tomato plants grown under different irrigation regimes and Ca-fertilization rates (Fig. 7b, c).

Discussion

Understanding the mechanisms involved in BER development is the key to effectively controlling this disorder in

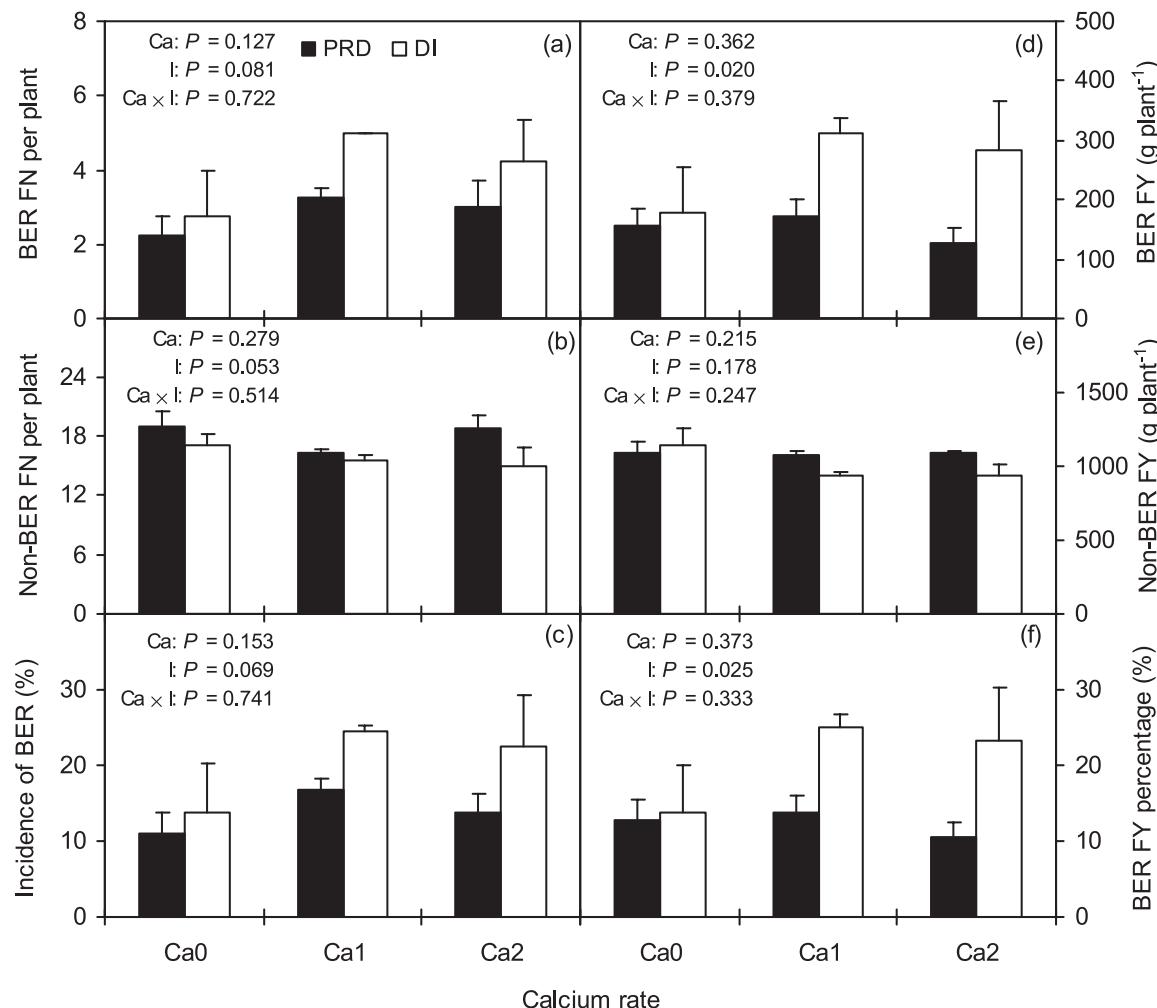


Fig. 3. BER fruit number (a), non-BER fruit number (b), BER incidence (c), BER fruit yield (FY) (d), non-BER fruit yield (E), and BER fruit yield percentage (f) of tomato plants under different reduced irrigation regimes (PRD and DI) and Ca-fertilization rates (Ca0, Ca1, and Ca2). Statistical comparisons (two-way ANOVA) between the irrigation and Ca-fertilization treatments as well as their interactions (Ca-fertilization, Ca; irrigation, I; and the interaction, Caxl) are presented for each variable. Error bars indicate SE ($n=4$).

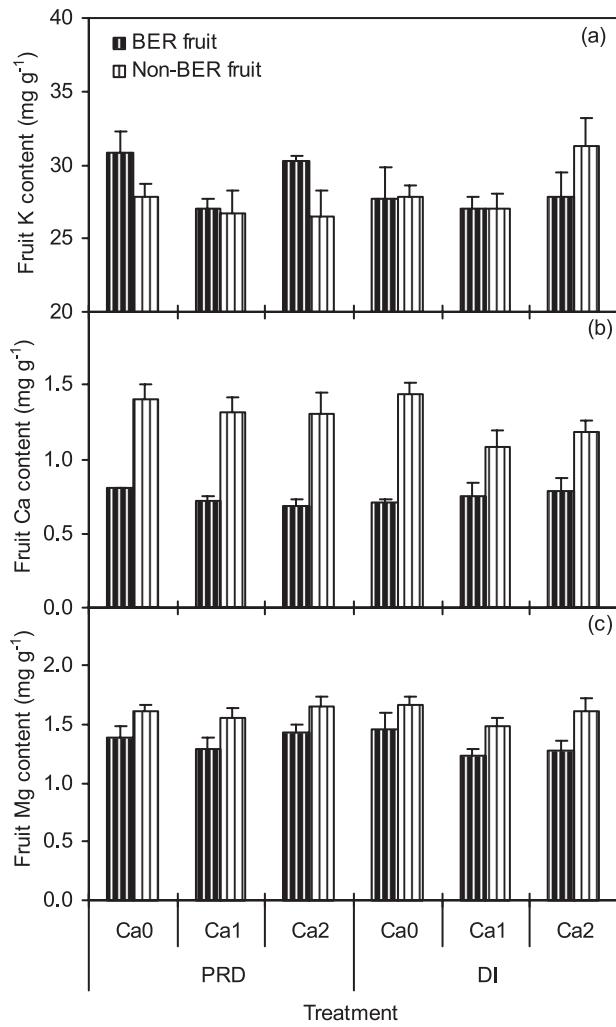


Fig. 4. K (a), Ca (b), and Mg (c) content in BER and non-BER fruits of tomato grown under different reduced irrigation regimes (PRD and DI) and Ca-fertilization rates (Ca0, Ca1, and Ca2). Statistical comparisons (two-way ANOVA) between the irrigation and Ca-fertilization treatments as well as their interactions (Ca-fertilization, Ca; irrigation, I; and the interaction, CaxI) are given in Table 2. Error bars indicate SE ($n=4$).

tomatoes (Abdal and Suleiman, 2005). Although there is evidence suggesting that Ca deficiency is neither a primary nor an independent factor in the development of BER (Nonami *et al.*, 1995; Saure, 2001), it is generally believed that inadequacy of Ca in the fruit is closely associated with BER

incidence in tomatoes (Ho and White, 2005). In accordance with this, here we observed that BER fruits had a significantly lower Ca content than non-BER fruits (Fig. 4b). In addition, it was found that the incidence of BER negatively correlated with the weighted average Ca content in the fruits (Fig. 7a), affirming an essential role of Ca deficiency in inducing BER (Ho and White, 2005). Most interestingly, when comparing the BER incidence rate and the BER fruit yield percentage between the two reduced irrigation regimes, it was found that PRD significantly reduced the BER fruit yield and tended to reduce BER incidence (Fig. 3).

In the literature, the cause of BER development in tomato has been attributed to a low Ca level in the whole plant due to decreased soil Ca supply or root Ca uptake, low transport of Ca to and in the fruit, or an increased demand for Ca due to a high growth rate of the fruit (Ho *et al.*, 1995; Saure, 2001; Ho and White, 2005). In the present study, the soil Ca supply was obviously not a factor limiting plant Ca status, as most of the measured variables, e.g. the Ca contents of different plant organs and the BER incidence, were unresponsive to the Ca-fertilization rates, despite the fact that the concentration of Ca^{2+} in the xylem sap was significantly affected (Table 1). Therefore, most of the observed significant effects of the treatments could have been caused by the two reduced irrigation regimes. This also supports the main hypothesis of the present study that different responses triggered by the PRD and DI brought about the distinguished effects on BER development in tomatoes.

Several mechanisms may be involved in lowering BER incidence in PRD compared with its counterpart DI treatment. Earlier studies have frequently observed that soil moisture stress can stimulate BER development (e.g. Adams and Ho, 1993), and reduced irrigation practices often result in a greater incidence of BER in tomatoes (Reid *et al.*, 1996; Franco *et al.*, 1999; Taylor *et al.*, 2004). It is well known that Ca movement in the plants and accumulation in the fruit are tightly linked with the transpiration rate of the plants (Keiser and Mullen, 1993). Basically, a decrease in plant transpiration rate could reduce plant total Ca uptake, and may thus increase BER occurrence in tomato (Taylor *et al.*, 2004) and sweet pepper (Tadesse *et al.*, 2001). However, as the leaf possesses a higher transpiration rate than the fruit, it often acts as a competing sink with the fruit for directional Ca flow and accumulation (McLaughlin and Wimmer, 1999; Taylor *et al.*, 2004). Accordingly, it has been suggested that a decreased leaf transpiration rate may actually reduce BER incidence

Table 2. Output of the two-way ANOVA for K, Ca, and Mg content in BER fruit, non-BER fruit, leaf, stem, and root of tomato plants as affected by the irrigation regimes (PRD and DI) and Ca-fertilization rates (Ca0, Ca1, and Ca2). The data is presented in Figs 4 and 5.

Factor	K content					Ca content					Mg content				
	BER	NBER	Leaf	Stem	Root	BER	NBER	Leaf	Stem	Root	BER	NBER	Leaf	Stem	Root
Irrigation	NS	NS	NS	NS	*	NS	NS	NS	NS	***	NS	NS	*	NS	NS
Ca-fertilization rate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Irrigation×Ca rate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*; ** Significant at $P < 0.05$ and $P < 0.001$, respectively; NS, not significant.

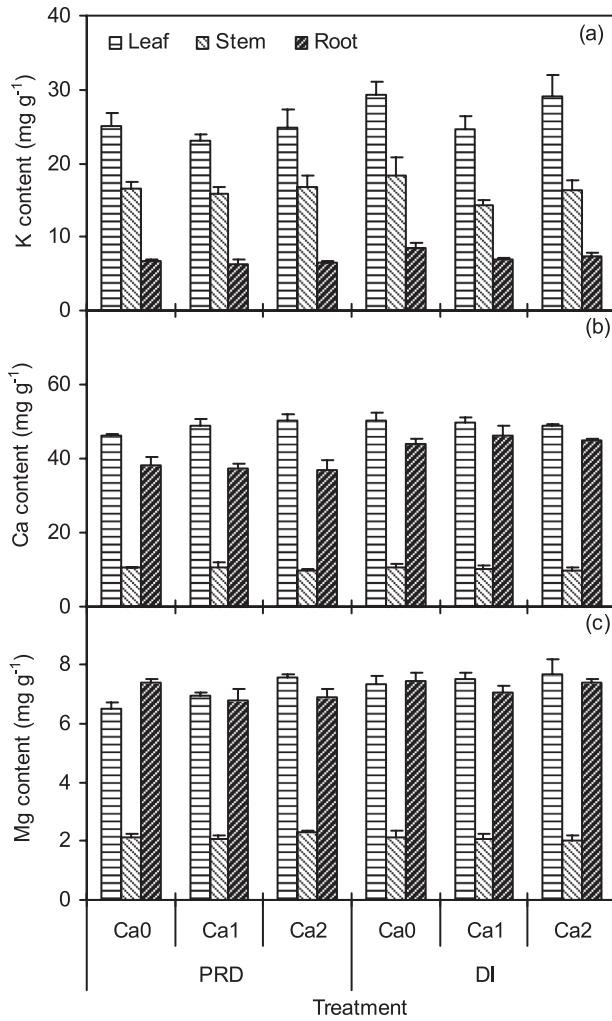


Fig. 5. K (a), Ca (b), and Mg (c) content in the leaf, stem, and root of tomato plants grown under different reduced irrigation regimes (PRD and DI) and Ca-fertilization rates (Ca0, Ca1, and Ca2). Statistical comparisons (two-way ANOVA) between the irrigation and Ca-fertilization treatments as well as their interactions (Ca-fertilization, Ca; irrigation, I; and the interaction, CaxI) are shown in Table 2. Error bars indicate SE ($n=4$).

in tomatoes (Li *et al.*, 2001; Ho and White, 2005). Likewise, increase fruit transpiration could also be an effective approach to increase fruit Ca uptake in tomatoes (Paiva *et al.*, 1998). In the present study, PRD plants exhibited significantly greater [ABA]_{xylem} than DI plants (Fig. 1d), which caused significantly lower g_s (Fig. 2) and resulted in a higher plant water status (Fig. 1a, b). These results are in good agreement with earlier findings in tomatoes under similar treatments (Dodd *et al.*, 2008; Wang *et al.*, 2010). Compared with the DI plants, the lowered g_s of the PRD plants, however, did not significantly reduce the total Ca uptake by the plants (data not shown). On the other hand, as has been mentioned previously in this paper, tomato fruits are hydraulically isolated from the shoot, and the xylem-borne ABA signal may affect the leaf physiology more significantly than the fruit (Davies *et al.*, 2000). Therefore, a reduced g_s and hence leaf transpiration rate in the PRD plants might have altered the pattern

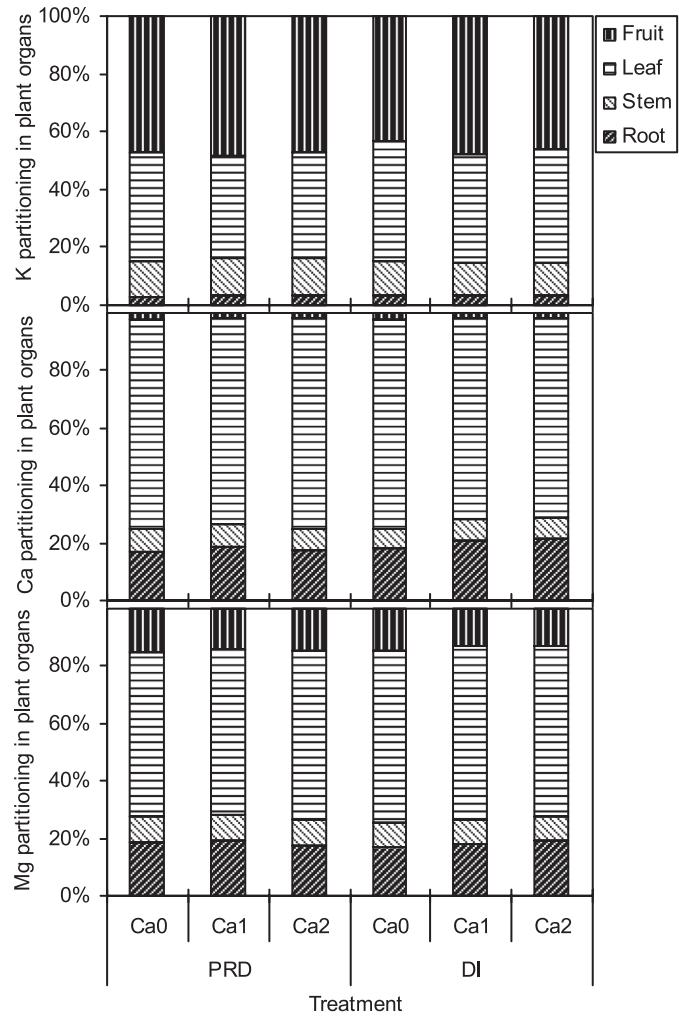


Fig. 6. Partitioning of K, Ca, and Mg among the fruit, leaf, stem, and root of tomato plants under different reduced irrigation regimes (PRD and DI) and Ca-fertilization rates (Ca0, Ca1, and Ca2). Statistical comparisons (two-way ANOVA) between the irrigation and Ca-fertilization treatments as well as their interactions (Ca-fertilization, Ca; irrigation, I; and the interaction, CaxI) are shown in Table 3.

of xylem water flux by increasing the proportion towards the fruit, which could consequently have enhanced Ca partitioning into the fruit, as observed here (Fig. 6).

It was noteworthy that a reduced Ca content and partitioning in the roots in the PRD compared with DI plants existed (Figs 5 and 6). Higher ABA and Ca contents in the roots have both been found to promote water flow across the root, contrasting with their effects on g_s (Quintero *et al.*, 1999). It has been proposed that, under stressful conditions, plants are able to effectively coordinate the rate of water uptake by roots and the rate of water loss through stomata, partially by modulating the ABA and Ca levels in the roots and shoots (Quintero *et al.*, 1999). Here, such coordination of water flux by ABA and Ca was seemingly evident for both DI and PRD plants. In the DI plants, the greater Ca content in the roots might have promoted root water uptake, which corresponded to the higher g_s and greater leaf transpiration rate of the plants,

Table 3. Output of the two-way ANOVA for K, Ca, and Mg partitioning in the fruit, leaf, stem, and root of tomato plants as affected by the irrigation regimes (PRD and DI) and Ca-fertilization rates (Ca0, Ca1, and Ca2). The data is presented in Fig. 6.

Factor	K partitioning				Ca partitioning				Mg partitioning			
	Fruit	Leaf	Stem	Root	Fruit	Leaf	Stem	Root	Fruit	Leaf	Stem	Root
Irrigation	NS	NS	*	NS	*	NS	*	*	*	NS	*	NS
Ca-fertilization rate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Irrigation×Ca rate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Significant at $P < 0.05$; NS, not significant.

whereas in the PRD plants, the higher $[ABA]_{xylem}$ had induced partial stomatal closure (Fig. 2), which could have reduced plant water consumption and root water uptake. However, the underlying mechanisms for the significant lower Ca partitioning towards the roots in the PRD compared with the DI plants remain unknown and merit further investigations.

In addition to lowering g_s , PRD significantly increased plant water status (i.e. Ψ_r), which may lower the hydrostatic tension in the xylem, allowing more water and Ca movement into the fruit compared with DI plants (Guichard *et al.*, 2005; Tonetto de Freitas *et al.*, 2011). In accordance with this, investigation on the cause of elevated BER incidence in tomato by osmotic or water stress has been attributed mainly to a reduction in Ca translocation into the fruit (Ho and White, 2005; Karlberg *et al.*, 2006; Magan *et al.*, 2008). Moreover, recent evidence demonstrates that exogenous ABA treatment could enhance the xylem connection between the shoot and the fruit and facilitate fruit water and Ca uptake (Tonetto de Freitas *et al.*, 2011). Although we did not determine the effects of the reduced irrigation regimes and Ca-fertilization rates on vascular tissue development in tomato fruits, based on the aforementioned findings (Tonetto de Freitas *et al.*, 2011), it is proposed that a higher abundance of functional xylem vessels may also be involved in the prevention of BER development in the PRD plants.

According to DeKock *et al.* (1982), the K:Ca ratio in the fruit is a better indicator for BER than Ca measurements alone. In

the present study, we noticed that there was a significant positive relationship between BER incidence and the K:Ca ratio in the fruit (Fig. 7c). However, Bar-Tal and Pressman (1996) found that the incidence of BER correlated better with the K:Ca ratio in the leaves than with the K:Ca ratio in the fruits. Nevertheless, a significant antagonistic effect of K on Ca uptake has frequently been observed in hydroponically grown tomatoes; for instance, an increase in K concentration in the nutrient solution often reduces Ca uptake, thus increasing the risk of BER development (Bar-Tal and Pressman, 1996; Wada *et al.*, 1996; Taylor *et al.*, 2004). Besides the K:Ca ratio, here we also found that the Mg:Ca ratio in the fruit positively correlated with BER incidence (Fig. 7b). Studies have indicated that there is a significant antagonistic effect of Mg on Ca uptake in tomatoes (Hao and Papadopoulos, 2004). These authors reported that when Ca concentration is low, increasing Mg concentration in the nutrient solution linearly increased the BER incidence in tomatoes. Collectively, these results indicate that, apart from fruit Ca content, the relative abundance of K and Mg to Ca in the fruit might play an important role in affecting fruit Ca uptake and thus BER incidence in tomatoes.

In conclusion, our results clearly demonstrated that, in comparison with DI treatment, PRD significantly reduced BER incidence in tomatoes. A greater $[ABA]_{xylem}$, lower g_s , and higher plant water status in the PRD than in DI plants might have contributed to the increased fruit Ca uptake, which could have reduced BER occurrence in the tomato

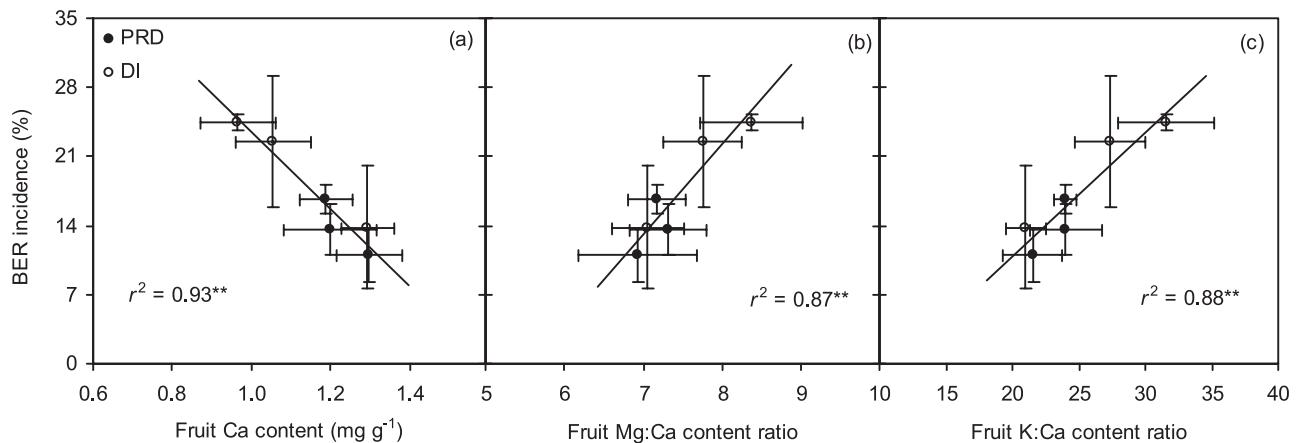


Fig. 7. Relationships between BER incidence and fruit Ca content (a), fruit Mg:Ca ratio (b), and fruit K:Ca ratio (c) of tomato grown under different reduced irrigation regimes (PRD and DI) and Ca-fertilization rates (Ca0, Ca1, and Ca2). ** and *** indicate the significance of the regression lines at $P < 0.01$ and $P < 0.001$, respectively.

fruits. Therefore, under conditions with limited freshwater resources, application of PRD irrigation could be a promising approach for saving water and preventing BER development in tomatoes.

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